Atlanto-Iberian (ICES areas, VIIIc, IXa) sardine spawning biomass estimation through the application of DEPM in 2011

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1. Background

The DEPM for estimation of sardine spawning biomass within the Atlanto-Iberian stock area is conducted every three years by IPIMAR (Instituto de Investigação das Pescas e do Mar, Portugal) and IEO (Instituto Español de Oceanografía, Spain) in an internationally coordinated survey, planned under the framework of ICES WGACEGGS and executed with co-financing from DCF. For this application, the ICES areas IXa and VIIIc are surveyed from Gibraltar (Gulf of Cadiz) to the Spanish/ French border in the north (Bay of Biscay).

In 2011, the Portuguese survey took place in February/March covering the Atlantic waters from the entrance of the Strait of Gibraltar to the northern border of Portugal, while the Spanish survey took place in March/April covering the northern stock area from the river Minho to the south of the Armorican shelf (in French waters).

This working document provides a description of the survey, laboratory analysis and estimation procedures used to obtain egg and preliminary adults parameters for the 2011 DEPM application to the Atlanto-Iberian sardine stock. Estimation of the spawning stock biomass (SSB) is also obtained for the northern stratum - northern coast of Spain.

Estimation was based on procedures and software adapted and developed during the WKRESTIM- 2009 and modifications carried out subsequently for the revision of the sardine DEPM historical series (1988-2008) in divisions IXa and VIIIc that is underway. Several issues that arose from processing, namely the choice of GLM model for the egg mortality curve fitting are planned for discussion at the WGACEGG prior to the obtainment of the final estimation to be send to WGHANSA A final SSB estimation will be estimated only once all the adult parameters are available.

2. Methodology

2.1 Surveying

The Portuguese DEPM survey was carried out from the 10th February to the 08th March onboard RV Noruega, while the Spanish survey was undertaken using two vessels, from the 25th March to the 10th of April onboard RV Cornide de Saavedra (for plankton sampling mainly) and from 26 March to 22 April using RV Thalassa to carry out the fishing hauls (Table 1).

Plankton sampling

During both surveys, vertical plankton hauls were carried out following a pre-defined grid of sampling stations along transects perpendicular to the coast and spaced 8 nmiles (Figure 1). The inshore limit of the transects was dependent on bottom depth (as close to the shore as possible), while the offshore extension was decided adaptively.

The main sampler for the DEPM is the PairoVET net that collects eggs through the water column at point stations. The PairoVET sampler (=double CalVET) includes 2 nets (Ø 25cm) with 150 µm mesh size and a CTDF probe; sampling covered the water column from bottom, or 150m (100 m for IEO) (beyond the 150 isobath) depth, to the surface. PairoVET samples were taken every 3 nm in the inner shelf (up to 200 m depth or 100 m where the platform is wider) and every 3 or 6 nm beyond the inner shelf, depending on the results of the CUFES sampler.

CUFES was used as the auxiliary egg sampler, helping in defining vertical hauls density and offshore extension of the transects. The outer limit of a transect was reached when two consecutive CUFES samples were negative beyond the 200 m depth.

All plankton samples were preserved in formalin at 4% in distilled water and the 2 samples from each net stored in separate containers. For IPIMAR both nets (Table 1) were used for egg density estimates while IEO used 1 net (the other being used for plankton dried mass calculations). IEO counted total number of eggs from the CUFES onboard in order to obtain a preliminary data of sardine egg abundance and distribution.

Temperature, salinity and fluorescence.

In the Portuguese survey, records of water temperature, salinity and fluorescence were obtained for surface waters by the CTF probes associated with the CUFES water pump; the CTDF profiler usually used together with the vertical nets was not available for the 2011 survey. A CTD (Sea Bird 37) profile (Temperature and Salinity) was carried out in each CalVET station in the Spanish survey. Morover a CTD (Sea Bird-25) was used in each transect head and in alternate stations along the transects.

Adult fish surveying

Fishing hauls were conducted by either pelagic or bottom trawling following sardine schools detection by the echo-sounder. The number of samples and its spatial distribution was organized to ensure good and homogeneous coverage of the survey area (Figure 2). In the Portuguese survey, the samples collected by the RV were complemented with samples obtained from commercial purse-seiners at Olhão, Portimão, Sines Setúbal, Peniche, Figueira da Foz and Matosinhos. Samples from the fishing fleet were acquired within 1-2 weeks of the surveying by RV Noruega in each area, except for 5 trawls (Matosinhos, Peniche, Portimão) which took place with about 1 month lag.

Onboard the RV, and for each haul, a minimum of 60 sardines were randomly selected and biologically sampled. These were, in some occasions, also complemented by additional fish in order to achieve a minimum of 30 females per haul for histology, and/or to obtain extra hydrated females for the fecundity estimations. Individual biological information (length, total weight, sex, maturity state, gonad weight) was recorded for all fish, the ovaries were preserved for histology (with a 4% formaldehyde solution diluted in distilled water and buffered with sodium phosphate) and the otoliths removed (only from females for IPIMAR) for age determination. The biological sampling and ovaries fixation were always carried out in fresh material, with the exception of 5 commercial samples for which the ovaries were removed from the fish body and preserved immediately after the fish were landed, while the remaining body of the fish was frozen for posterior biological sampling in laboratory.

2.2 Laboratorial analyses

Plankton samples

In the laboratory, all sardine eggs were sorted from PairoVET and CUFES samples. The eggs from the vertical hauls (2 nets – IPIMAR, 1 net –IEO) were all counted and staged according to the 11 stages of development classification (adapted from Gamulin and Hure, 1955). For IPIMAR, the eggs from the CUFES sampler were all counted and a sub-sample, of a minimum of 100, was staged per sample.

Adult fish samples

The preserved ovaries were weighed in laboratory and the obtained weights corrected by a conversion factor (between fresh and formaldehyde fixed material) established previously. These ovaries were then processed for histology: they were embedded in either resin (IEO) or paraffin (IPIMAR), the histological sections were stained with haematoxylin and eosin, and the slides examined and scored for their maturity state (most advanced oocyte batch) and POF presence and age (Hunter and Macewicz 1985, Pérez et al. 1992a, Ganias et al. 2004, Ganias et al. 2007). Prior to fecundity estimation, hydrated ovaries were also processed histologically in order to check for POF presence and thus avoid underestimating fecundity (Pérez et al. 1992b). The individual batch fecundity was then measured, by means of the gravimetric method applied to the hydrated oocytes, on 1-3 whole mount sub-samples per ovary, weighing on average 50-150 mg (Hunter et al. 1985).

2.3 Data analyses

Temperature and salinity

A comparison at 10m depth between the information registered by the SBE-25 (major precision) and SBE-37 (minor resolution) in the IEO survey was carried out in order to assess the error produced by working with a CTD with minor resolution in each CalVET station (SBE-37).

Egg data

All calculations for area delimitation, egg ageing and model fitting for egg production (P_0) estimation were carried out using the R packages (*geofun, eggsplore and shachar*) available within the open source project *ichthyoanalysis* (http://sourceforge.net/projects/ichthyoanalysis). Some routines of the R packages used were updated since the 2008 versions.

To avoid high and low extremes values detected in the area represented by each of the sampled stations, this values were forced to the minimum and maximum values of 25 and 175 respectively (the extreme values usually occur on the borders of the survey area and therefore do not affect the estimation of the positive area). The range 25-175 was selected to be a mean interval suitable according to the distance between transect and stations (fixed to be 8 nm between transects and 3 between stations along the transects).

The model of egg development with temperature was derived from the incubation experiment data available within the *sardata* R library. Egg ageing was achieved by a multinomial Bayesian approach described by Bernal *et al.* (2008) and using *in situ* SST. Distribution of the daily spawning cycle was assumed as a normal (Gaussian) distribution, with a peak at 21:00 h GMT and a standard deviation of 3 h (spawning period form 21-6 h to 21+6 hours). It is assumed that 0 time is at midnight and days are 24 hours long. The options used for the depm.control parameters were in agreement with the decisions undertaken during the 2009 WGACEGG meeting. Peak spawning time was taken from the literature (Bernal, 2008) and the spawning curve considered in order to be more conservative and allow a longer spawning period that few eggs were excluded from the analyses (how.complete=0.99). The upper age cutting limit was determined using a maximum age for the strata considered and it not dependent on the individual stations (upper.age=F). The lower age cutting excluded the first cohort of stations in which the sampling time is included within the daily spawning period (lower.age=T).

Three different sets of strata were used in the analysis; some based on previous analyses and others reorganized in order to estimate mortality and/or egg production. The strata were defined according to biological/ecological (geographical) reasons (see Bernal *et al.*, 2007) and also to consider timing of survey (IPIMAR and IEO surveys are on average 1 month apart)

- No strata: unique strata for all Atlanto-Iberia, from the strait of Gibraltar to the Spanish-French Atlantic limit.
- Three strata (Stratum); **South**, encompassing from the strait of Gibraltar to Cape St. Vicente, **West**, from Cape St. Vicente to the northern limit between the Spain and Portugal, and **North**, between the Spanish-Portuguese northern limit and the Spanish-French Atlantic limit.
- Two strata (StratumI); **South-West**, encompassing the Gulf of Cádiz and the Western Iberian coast up to the northern Portuguese Spanish limit (stratum south and west above), which includes the area covered by the Portuguese survey, and **North**, which coincides with the northern stratum defined above, the Spanish survey.

The second set of strata represent the current view of the different nuclei of the stock (Bernal *et al.*, 2007, Silva, 2007), while the third set of strata represent the area covered by the Portuguese and Spanish survey respectively, which were carried out with seventeen days difference. Then, a series of tests were carried out in which estimates of mortality and/or egg production were aggregated first into the two strata of the third set and then into a unique estimate for all Atlanto-Iberia. The final model was selected using a combination of significance of the mortality estimates.

The maximun age and temperature was calculated for the different strata described previously. Estimates of egg production and mortality were initially estimated for the entire area (no strata), for each stratum of the second set and for the two strata of the third stratum set.

The exponential model: E [P] = P0 e $^{-Z\ age}$ was fitted by a Generalized Linear Model (GLM), assuming a negative binomial distribution. Finally, the total egg production was calculated multiplying the daily egg production by the positive area (area with eggs defined by an automated procedure using the spatstat library)

The models used to estimate mortality and egg production were:

Model 1
<u>1 strata and 1 mortality</u>
glm.nb(cohort ~ offset(log(Efarea)) + age, weights=Rel.area, data=aged.data)

Model 2
<u>3 strata (Stratum) and 3 mortalites (Stratum:age)</u>
glm.nb(cohort ~ offset(log(Efarea)) -1 + Stratum+ Stratum:age, weights=Rel.area, data=aged.data)

Model 3
<u>3 strata P0 (Stratum) and 1 mortality (age)</u>
glm.nb(cohort ~ offset(log(Efarea)) -1 + Stratum+ age, weights=Rel.area, data=aged.data)

Model 4
<u>3 strata P0 (Stratum) and 2 mortalities (StratumI:age) (1z for IPIMAR, 1z for IEO)</u>
glm.nb(cohort ~ offset(log(Efarea)) -1 + Stratum+ StratumI:age, weights=Rel.area, data=aged.data)

Model 5
<u>2 strata (StratumI) e 2 mortalidade (StratumI:age)</u>
glm.nb(cohort ~ offset(log(Efarea)) -1 + StratumI+ StratumI:age, weights=Rel.area, data=aged.data)

Fish data

The adult parameters estimated for each fishing haul considered only the mature fraction of the population (determined by the fish macroscopic maturity data) and was based on the biological data collected from both surveys and commercial samples. Before the estimation of the mean female weight per haul (W), the individual total weight (Wt) of the hydrated females was corrected by a linear regression between the total weight of non-hydrated females and their corresponding gonad-free weight (Wnov). The sex ratio (R) in weight per haul was obtained as the quotient between the total weight of females on the total weight of males and females. The expected individual batch fecundity (Fexp) for all mature females (hydrated and non-hydrated) was estimated by modelling the individual batch fecundity observed (Fobs) in the sampled hydrated females and their gonad-free weight (Wnov) by a GLM. In case a geographical variability was observed in individual batch fecundity, a posterior post-stratification was carried out, Fobs being modelled against the Wnov and the Stratum (second and third sets of strata used for the egg data analysis). The fraction of females spawning per day (S) was determined, for each haul, as the average number of females with Day-1 or Day-2 POF, divided by the total number of mature females (the number of females with Day-0 POF was corrected by the average number of females with Day-1 or Day-2 POF, and the hydrated females were not included) (Pérez et al. 1992a, Ganias et al. 2007). The mean and variance of the adult parameters for all the samples collected was then obtained using the methodology from Picquelle and Stauffer 1985 (weighted means and variances). All estimations and statistical analysis were performed using the R software. Final adult parameters include individual estimates for the Southern, Western and Northern areas, with three independent estimates.

Details on the methodologies used on board, during laboratorial work and for data analyses are summarized in Table 1.

3. Results

Temperature and salinity

Surface temperature and salinity distributions are presented in figure 3. Temperature values ranged from 12.5 to 16.9 ºC and the distribution patterns were similar to observations from previous years; the highest temperature values were observed in the southern area and the lowest values registered for the Cantabrian Sea. The winter/spring conditions in the Atlanto-Iberian region were very unstable and much severe than in 2008. During the first quarter of 2011 heavy rain and strong winds were frequent.

For the area covered by the Portuguese survey the temperature data used for egg ageing was registered underway at 3m depth (CTF probe). During the Spanish survey the data were extracted from the SBE-25 and SBE-37 records The comparison of the results collected at 10m showed small differences between the information registered by the SBE-25 (major precision) and the information interpolated from the SBE-37 (minor precision) data. For the majority of the cases (> 90 %) the differences were lower than 0.5ºC, and were lower than 0.2ºC for 75% of the values compared. Comparison for salinity showed differences between the readings from the two CTDs that were lower than 0.02 units, for 76 % of the values compared.

Eggs

In total 224 PairoVET hauls and 829 CUFES samples were obtained (Table 2), during the Portuguese survey the number of CUFES stations was reduced in circa 20% due to irreparable damage to the system. The percentage of stations with sardine eggs was 27% for the vertical tows and 33% for the surface samples. Considering only one of the PairoVET nets 3300 sardine eggs were gathered in total, of which more than half came from the northern region, around a third from the south and less than 15% from W Portugal The highest egg abundance per haul was 4950 reached in the South, while the lowest egg abundance per haul was 833 on the est coast. The distribution of eggs per stage included 51.9% of stage III, 45% of stage IV and 35.5 of stage II for South, West and North areas respectively (Figure 4). For the total area surveyed stage III eggs were the more abundant (27.8%)

Sardine egg distribution, obtained from the PairoVET and CUFES systems, for the whole area is presented in Figure 1. The egg distribution pattern derived from the observations from the two samplers is similar and it is evident that the area occupied by eggs was much smaller than in 2008, this is particularly clear for the West coast of Portugal. Higher egg densities were observed in the eastern regions of the Gulf of Cadiz and Cantabrian Sea

The surveys covered a total area of 83508 km2 of which 23745 km^2 (28.4 %) were considered the spawning area (Table 3). The northern stratum represented 52.5 % of the spawning area while 27.5 % were in the southern coast and 20.3 % in the western shores

Table 3 shows the mortality values obtained using geographical stratification (no strata, 3 strata and two strata) as described above. Mortality values for the southern and western regions are much higher than for the northern stratum. Mortality calculated for each one of the three strata defined shows negative and significantly different from zero values and was considered acceptable for egg production estimation. Egg mortality is very much dependent on water temperature. When mortality is considered by geographic stratum the values estimated decrease from south to north, higher temperatures shorten egg duration and usually give rise to an increase in the estimates of mortality. Conversely, lower temperatures, more common in the north, originate slower egg development and lower mortality. Therefore choice of GLM model, with one, two or three slopes (mortality), may give distinct results for the egg production (intercept) by stratum.

Final egg production models (Table 3 and Figure 5) include individual egg production estimates for the Southern, Western and Northern areas, with three independent mortality estimates (Model 2), three egg productions with a common slope for the the whole Atlanto Iberian stock (Model 3) and two separate mortality estimates (one for the South and West combined, and one for the Northern area- Model 4). With model 5 estimates of egg production and mortality are obtained for the two surveys (IPIMAR and IEO) and finally, egg production with a single mortality, estimated for the whole Atlanto Iberian stock, is considered using Model 1.

The summaries of the five different models used to estimate egg production are shown in Annex 1.

Although the results from different GLM models could be considered an option for the final egg production estimation (negative and statistically significant mortality), large differences in the estimates by areas are introduced due to the choice of model used.

The final model to estimate egg production should be selected according to a combination of the following criteria:

- obtainment of a negative (and significant) value for mortality estimates
- P0s estimates for 3 strata (to improve detail for the P0, that may allow better description of processes than when considering only one strata for all the Atlanto-Iberian stock)
- knowledge on the biology/ecology of the species and system in the distinct areas surveyed

For all models, daily egg production per m^2 (eggs/m2/day) is highest for the southern region.

Total egg production (eggs/day) estimated for the Atlanto Iberian stock varies from 7.01×10^{12} (model 1) to 8.29×10^{12} (model 4). Using three P0s and three mortality estimates (Model 2), the added total egg production estimate was 7.33 $x10^{12}$; 3.83 $x10^{12}$ corresponding to the south, 1.81 $x10^{12}$ to the west and 1.69 $x10^{12}$ to the north.

Adults

For the 2011 survey an effort was made to guarantee the level of sampling already achieved in the 2002, 2005 and 2008 surveys, however a high percentage of fishing hauls (48 %) over the total, resulted negative for sardine,. On the whole, 34 fishing hauls which caught sardines were performed during the surveys covering the whole area, complemented by 24 samples obtained from the Portuguese purse-seine fleet (Figure 2). On the whole, almost 3760 sardines were sampled (Table 2), more than 1450 ovaries were collected, preserved and analysed histologically and *ca.* 1070 otoliths were removed for age determination. A total of 72 hydrated females were caught for batch fecundity estimation (much lower than in 2008).

All laboratory tasks are completed for the samples collected during IEO's survey whereas the weighing in laboratory of the preserved ovaries sampled during IPIMAR's survey and from the Portuguese commercial fleet as well as their histological processing and microscopical analysis are still in progress. Therefore, spawning fraction (S) could presently be estimated for the Iberian Northern area (North strata) only, being unavailable for the Portuguese and Cadiz coasts (West and South strata). The other three parameters (W, R, F) calculated for strata 1 and 2 (South and West Portuguese coasts) were based on 29 of the 48 samples collected in this area, the present results being thus preliminary.

Data were analysed and the parameters estimated for the two surveys jointly:

- The same linear regression between the non-hydrated females Wt and their corresponding Wnov was used for the whole surveyed area (Wt = 1.073 * Wnov - 1.571, R^2 = 0.995).
- The geographical distribution of female weight (Figure 6) and mean observed batch fecundity (Fobs = 10619, 10218 and 39262 eggs/female, respectively, for South, West and North strata) suggest the need for a spatial stratification in view of the parameters estimation. However, considering that few hydrated females were collected off the South and West strata separately, the data from these two strata were pooled for the modelling of batch fecundity. Fobs data were thus modelled against the Wnov and the Stratum (GLM: Fobs ~ Wnov:Stratum, Normal errors distribution and identity link) with two different strata, South and West strata together and North stratum (Figure 7).
- Compared to previous DEPM years, the number of hydrated females collected was particularly reduced. In order to confirm the accuracy of the relationship between Fobs and Wnov and of its spatial variability, it is suggested that additional observed batch fecundity estimates be obtained using the gravimetric method applied to non-hydrated females (e.g., at the oocyte migratory nucleus stage; Ganias et al. 2010). This additional task could be performed with IPIMAR's samples only after the histological analysis of the ovaries has been completed.

The minimum mean weights by haul were observed in the North of Portugal and in the Gulf of Cadiz (Figure 6). Mean female weight (W) was similar for the whole Portuguese and Cadiz coasts (55.8 and 54.0 g for strata 1 and 2, respectively) and considerably higher for the Northern Spanish coast (81.4 g for stratum 3). Compared to previous surveys, mean female weight for the whole area surveyed was similar to the values estimated for the 2008 survey, the two latest surveys presenting the highest values of the historical series.

The mode of individuals age distribution off the Northern Spanish coast is 3 years-old, these fish representing almost half of the individuals for which otoliths were sampled. On the contrary, female age distribution is bimodal off the Western Portuguse and Southern coasts, with sardines aged 1 and 6 and over being the most abundant in the samples representing respectively, about one third and one quarter of the females for which otoliths were collected (the latter likely still corresponding to the 2004 strong recruitment) (Figure 6).

The geographical distribution of adults' parameters (off the SW and the N Iberian coasts) suggested the usefulness of spatial stratification for batch fecundity estimation, and the model obtained with the factor "Stratum" was statistically significant. The relationship between the Fobs and the female Wnov indicated that for the same fish weight, batch fecundity was considerably higher for the females sampled off the Northern Spanish coast than for the Portuguese and Cadiz areas, suggesting that relative fecundity was in 2011 lower for the latter (Figure 7). Though similar mean Fobs values were obtained between the West and South strata, the low number of observed batch fecundity data from IPIMAR's survey used for the analysis (n = 30) prevents to accurately determine if significant spatial differences exist between these two areas surveyed by IPIMAR.

Mean batch fecundity estimates (F) were considerably lower (one third) off the Portuguese and Cadiz than off the Northern Spanish coasts. The latter presented the highest estimate of the historical series, though similar to the ones obtained for the 2005 and 2008 surveys. On the contrary, F estimates from the Portuguese and Cadiz areas were the lowest of the time series, only comparable to the batch fecundities obtained for the 2002 survey. For the Southern and Western strata, although mean female weights were similar to the ones obtained during the 2008 survey, batch fecundity estimates in 2011 were reduced by a half when compared to the values obtained in 2008. Spawning fraction estimate for the Northern Spanish coast was higher than the one obtained during the 2005 and 2008 surveys (0.120 *vs.* 0.06 and 0.09, respectively).

SSB estimate

In the present document SSB was only estimated for the Northern area. It is important to note that large differences in the SSB estimate are found according to the value obtained for the selected model of total egg production. Using the egg results from model 2 (3 P0, 3 mortalities) the SSB estimated was 52.96 (thousand tons), from model 3 (3 P0, 1 mortality) estimated was 116.93 thousand tons and form model 4 (3 P0, 2 mortalities) 53.30 (Table 3).

Final remarks Despite the fact that the 2008 DEPM results for egg production and SSB were the highest of the historic series no strong recruitment has been identified in the past six years. In fact other sources such as acoustics surveying have been noticing a decline in the Iberian sardine since 2006 (ICES, 2011). During the 2011 survey the difficulty in obtaining positive hauls for sardine, even though considerable fishing effort was undertaken, suggests that the species was much less available than it was in 2008.

The spawning area estimates for 2011 are lower in all the strata compared to 2008 (ICES, 2009). The spawning area of sardine in the western and northern areas is much smaller than in 2008, around 75 and 50 % respectively.

Total egg production estimates in all areas are lower than in 2008 when estimates are based in a model with three different mortalities values (one mortality value for each area, Model 2). When egg production estimates are compared with those obtained using the same procedure carried out in 2008 (Model 4, three egg production and two mortalities values, one for southern and western areas and one for northern area), northern area egg production is higher than in 2008, but southern and specially western area egg production values are much lower than in 2008.

Mean female weights obtained for all strata were similar to the ones estimated in 2008, the values calculated for the N and NW coasts of Spain being higher than for the West and South strata.

As in previous years, batch fecundity estimates are considerably higher for the North than for the West and South strata in 2011. Nevertheless mean batch fecundity for the West and South strata showed the lowest values from the whole historical series, suggesting a decrease of female relative fecundity.

The spawning fraction for the North strata in 2011 is higher than in the two previous surveys.

SSB estimation for the North strata in 2011 is quite similar to those obtained in 2005 and 2008 when the model selected for the egg production estimate include a common mortality value for the three strata (model 3). On the contrary, using egg production from models 2 or 4 the SSB estimation for the North stratum decreases by about 50% when compared to the 2008 results.

A revision of the time series of DEPM-based SSB estimates is reported at WGACEGG 2011 and it will be considered for the benchmark assessment for Atlanto-Iberian sardine that will take place in 2012. Further comparison of results will therefore be completed when the revision is finalized.

Summary :

- spawning area in 2011 reduced compared to 2008
- spawning area in the western area much smaller than in 2008 (only around 20% of the
- total spawning area in 2011)
- the southern region showed the highest daily egg production per m_2 (eggs/m2/day)
- total egg production in all regions lower than in 2008 but higher than in 2002
- total egg production for 2011 was higher than in 2005 for S but lower for W and N
- main differences in total egg production between 2011 and 2008 were related to
- spawning area differences; reduced in all regions
- mortality values for S and W much higher than for N and higher than in 2008
- mean female weights similar to the ones obtained in 2008, higher for the North than for the West and South strata
- mean batch fecundity considerably higher for the North than for the West and South strata in 2011
- mean batch fecundity for the West and South strata among the lowest of the historical series
- spawning fraction for the North strata in 2011 higher than in the two previous surveys
- to note that comparison to the historic series are dependent on the revision that is underway to be presented at WGACEGG-2011

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Table 1. Surveying, processing and analyses for eggs and adults

DEPM Surveys	Portugal (IPIMAR)	Spain (IEO)
Survey	**PT-DEPM11-PIL**	**SAREVA0411**
Survey area	South-West	NW & N Spain
SURVEY EGGS		
Sampling grid	8 (transect) x 3(station)	8 (transect) x 3(station)
PairofVET Eggs staged (n egg) (stages from Gamulin and Hure, 1955)	All (2 net)	All (1 net)
Sampling maximum depth (m)	150	100
Temperature for egg ageing	10 m	
Peak spawning hour	(PDF 21 ± 2 * 3)	
Egg ageing	Bayesian (Bernal et al, 2008)	
Strata	No strata/Stratum (South,West,North)/StratumI (South+West/North)	
Egg production	GLM	
CUFES, mesh 335	3nm (sample unit)	3 nm (sample unit)
CUFES Eggs counted	All	All
CUFES Eggs staged (stages from Gamulin and Hure, 1955)	Subsampled of a minimun of 100	No
Hydrographic sensor	CTDF (FSI)	CTD (SBE 37) CTD SBE 25
Flowmeter	Y	Y
Clinometer	Y	Y
Environmental data	Fluorescence, Temperature, Salinity	Fluorescence (surface only), Temperature, Salinity
SURVEY ADULTS		
Biological sampling:	On fresh material, onboard the R/V or in laboratory; on frozen material for certain commercial samples (ovaries removed before)	On fresh material, on board of the R/V
Sample size	60 indiv randomly ; extra if needed (30 females min for histology) and if hydrated females found	60 indiv randomly (30 mature female); extra if needed and if hydrated found
Sampling for age	Otoliths from the same females sampled for histology	Otoliths from random males and females
Fixation	Buffered formaldehyde 4% (distilled water)	Buffered formaldehyde 4% (distilled water)
Preservation	Formalin	Formalin
Histology:		
- Embedding material	Paraffin	Resin
- Stain	Haematoxilin-Eosin	Haematoxilin-Eosin
S estimation	Day 1 and Day 2 POFs (according to Pérez et al. 1992a and Ganias et al. 2007)	Day 1 and Day 2 POFs (according to Pérez et al. 1992a and Ganias et al. 2007)
R estimation	The observed weight fraction of the females	The observed weight fraction of the females

F estimation	On hydrated females (without POFs), according to Pérez et al. 1992b and Ganias et al. 2010	On hydrated females (without POFs), according to Pérez et al. 1992b

Table 2. General Sampling DEPM 2011

Institute	**IPIMAR**	**IPIMAR**	**IEO**
Survey area	**South**	**West**	**NW & N Spain**
SURVEY EGGS			
R/V	Noruega	Noruega	Cornide de Saavedra
Date	10/02-20/02	20/02-08/03	25/03-10/04
Transects	21	36	56
PairoVET stations	170	309	337
Positive stations	54	40	130
Tot. Eggs	2208	803	1794 (1 net)
Max eggs/m2	4950	2970	1537
Temp (ºC) min/mean/max	14.6/16/16.9	13.5/14.7/16.1	12.5/13.4/14.6
Max age	56.8	66	70.9
CUFES stations	183	309	337
Positive CUFES stations	60	54	163
Tot. Eggs CUFES	4607	479 (inc. area)	34438
Max eggs/m3	81.73	22.13	97.26
Hydrographic stations	NA	NA	337
SURVEY ADULTS			
Number Hauls R/V (total)	11	23	53
- Pelagic Trawls	10	20	53
- Bottom trawls	1	3	-
Numer Hauls Commercial vessel	7	17	-
Number (+) trawls	16	32	10
Date	10.02-20.02	20.02-08.03	12/04-20/04
Depth range (m)	33-107	25-116	61-185
Time range	During the whole day		07:00-20:00
Total sardine sampled	975	2065	718
Length range (mm)	115-266	120-246	162-256
Weight range (g)	11-89	12-98	26.8-130.8
Female for histology	397	827	230
Hydrated females	11	30	31
Otholites	235	429	409
Female Ages Range	1-10	1-10	1-11

Table 3. Results DEPM 2011

Institute	IPIMAR	IPIMAR	IEO	TOTAL
Area	**South**	**West**	**NW & N Spain**	
Survey area (Km^2)	17577.99	32098.44	33831.56	83507.99
Positive area (Km^2)	6523.47	4816.68	12404.84	23744.99
Z (hour^{-1})(CV%)				
Model 1	-0.044*** (15)			
Model 2	-0.056***(21)	-0.073***(16)	-0.02**(31)	
Model 3	-0.045***(15)			
Model 4	-0.066***(14)		-0.02**(30)	
Model 5	-0.062 (15)		-0.02**(31)	
P0 (eggs/m2/day)(CV%)				
Model 1	295.4 (23)			
Model 2	587.4(41)	375.6 (44)	136.3(26)	
Model 3	418.4(27)	169.7(30)	300.9(25)	
Model 4	789.1(33)	299.3(35)	137.2(26)	
Model 5	519.7(31)		137.1(26.4)	
Daily mortality rate (%)				
Model 2	73.9	82.7	38.1	
Model 3	66			
Model 4	79.5		38.1	
Model 5	77.4		38.1	
P0 tot (eggs/day) (x10^{12}) (CV%)				
Model 1	7.01(23)			
Model 2	3.83(41)	1.81(44)	1.69(26)	7.53
Model 3	2.73(27)	0.82(30)	3.73(25)	7.28
Model 4	5.15(33)	1.44(35)	1.70(26)	8.29
Model 5	5.89(31)		1.70(26)	0.76
Female Weight (g)				
Three strata (S, W and N)	55.770 (9)	54.040 (7.1)	85.170 (3.6)	
Two strata (S+W, N)	54.650 (5.5)		85.170 (3.6)	
Batch Fecundity				
Three strata (S, W and N)	13009 (14.8)	12704 (8.2)	40169 (5.1)	
Two strata (S+W, N)	12812 (7.5)		40169 (5.1)	
Sex Ratio				
Three strata (S, W and N)	0.509 (13)	0.500 (6.3)	0.564 (13.7)	
Two strata (S+W, N)	0.503 (6)		0.564 (13.7)	
Spawning Fraction				
Three strata (S, W and N)	NA	NA	0.120 (26.2)	
Two strata (S+W, N)	NA		0.120 (26.2)	
Spawning Biomass (thousand tons) (CV%)				

Model 2			52.96 (40.1)	
Model 3			116.93 (39.1)	
Model 4 & 5			53.30 (36) (40.2)	

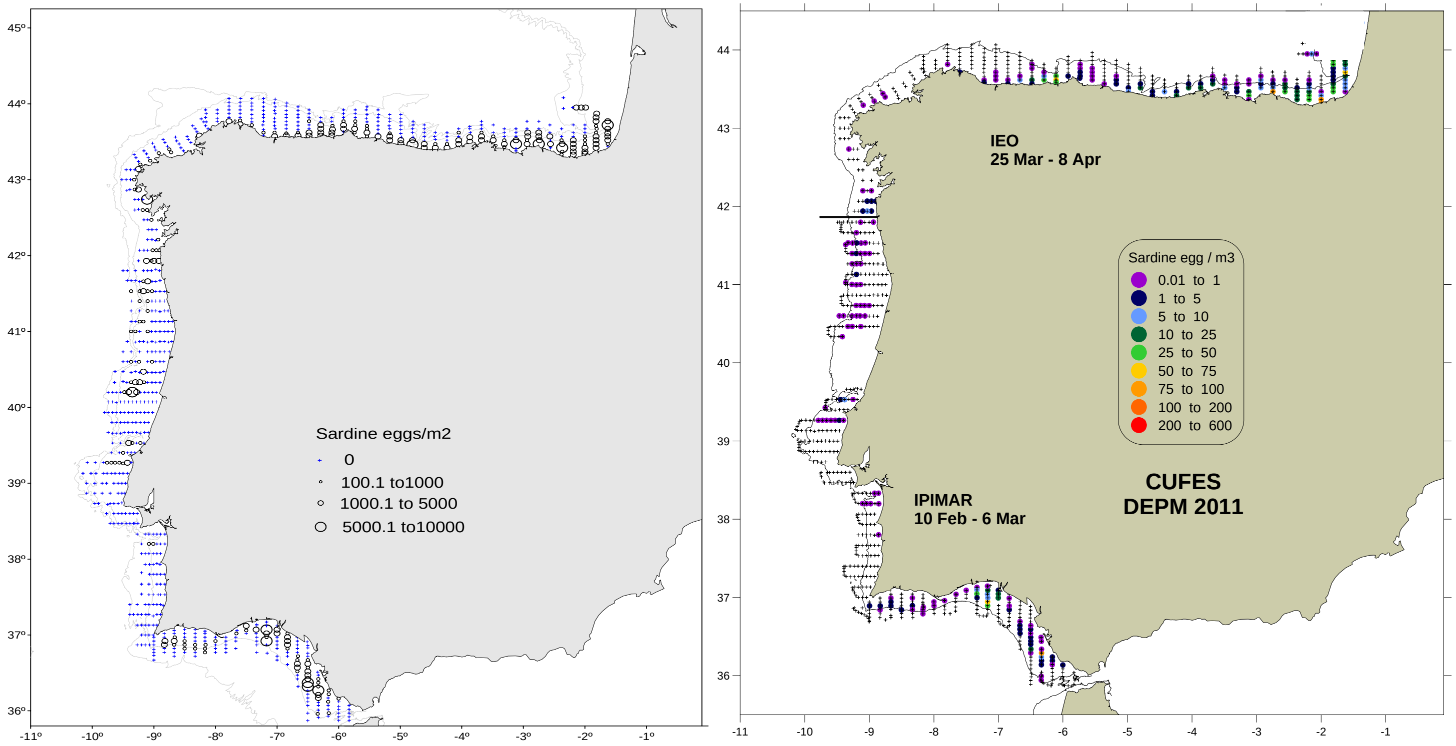


Figure 1. Sardine egg distribution. Left panel: Egg/m^2 from PairoVET sampling; Right panel: Egg/m^3 from CUFES sampling; (+, egg absence).

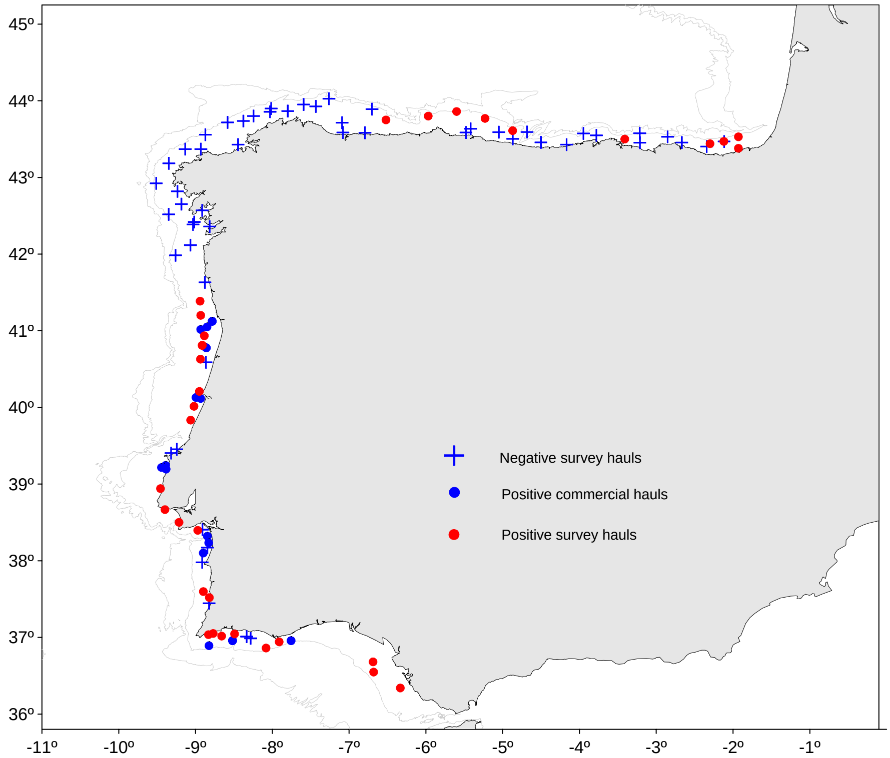


Figure 2. Spatial distribution of fishing hauls (+, hauls without sardine presence)

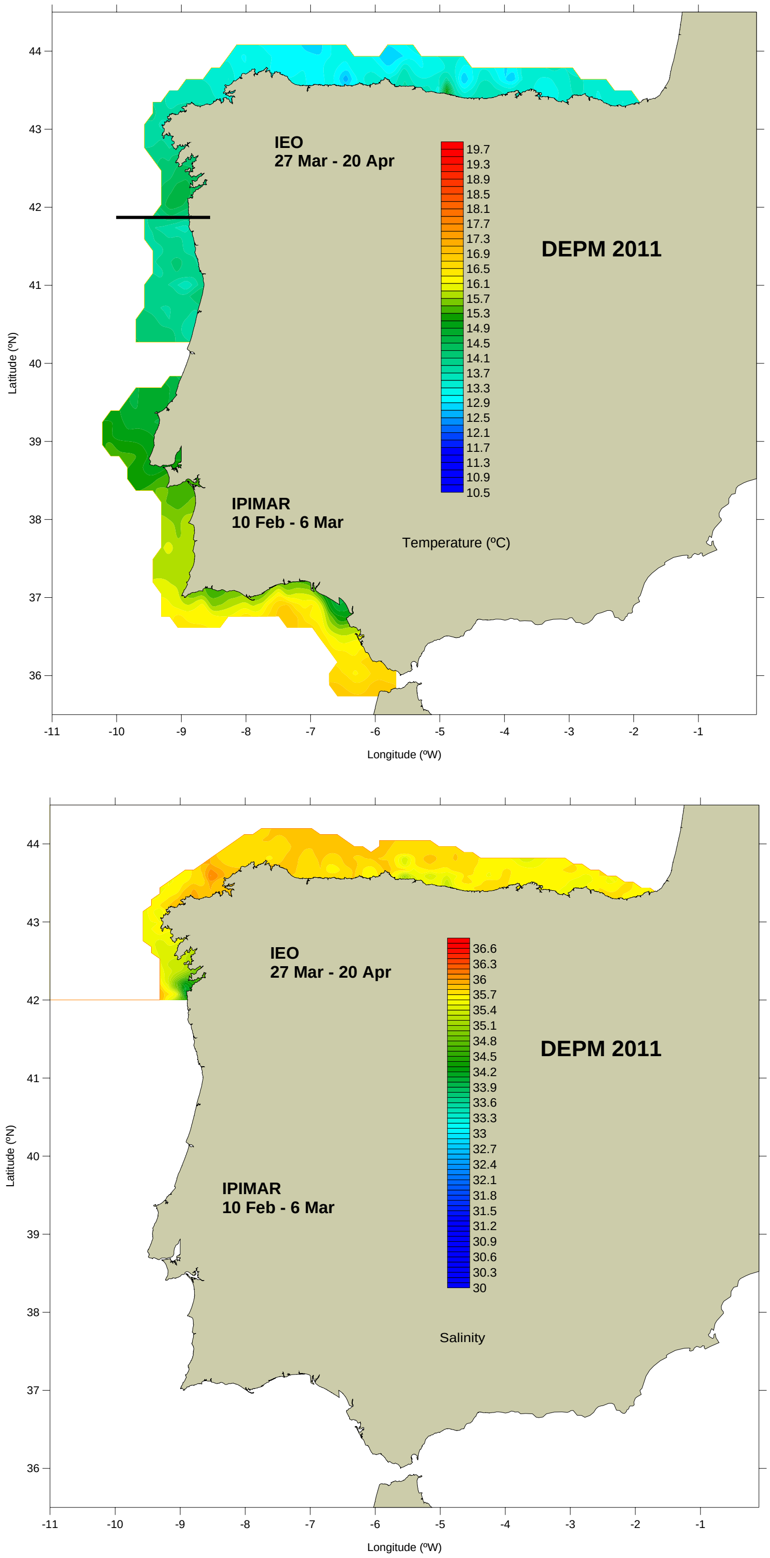
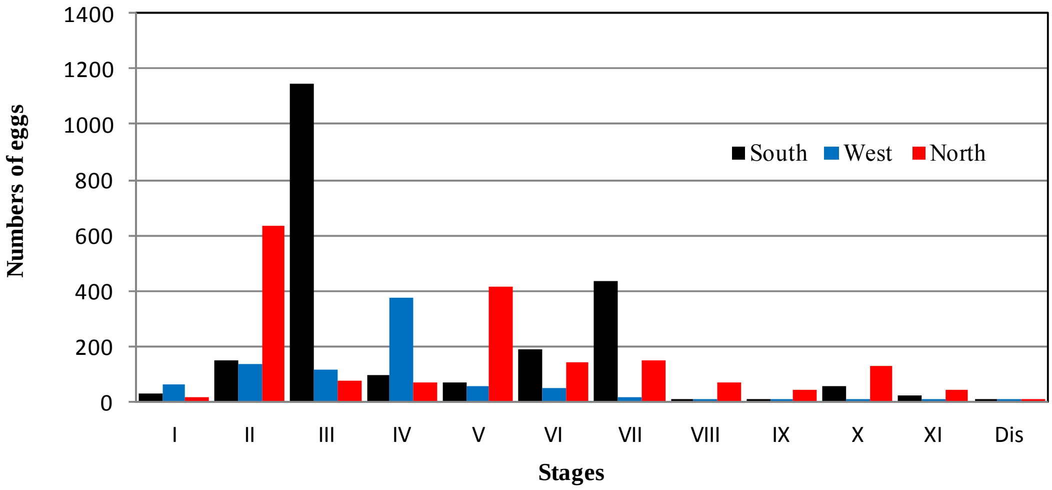


Figure 3. Distribution of sea surface temperature (above) and salinity (below).



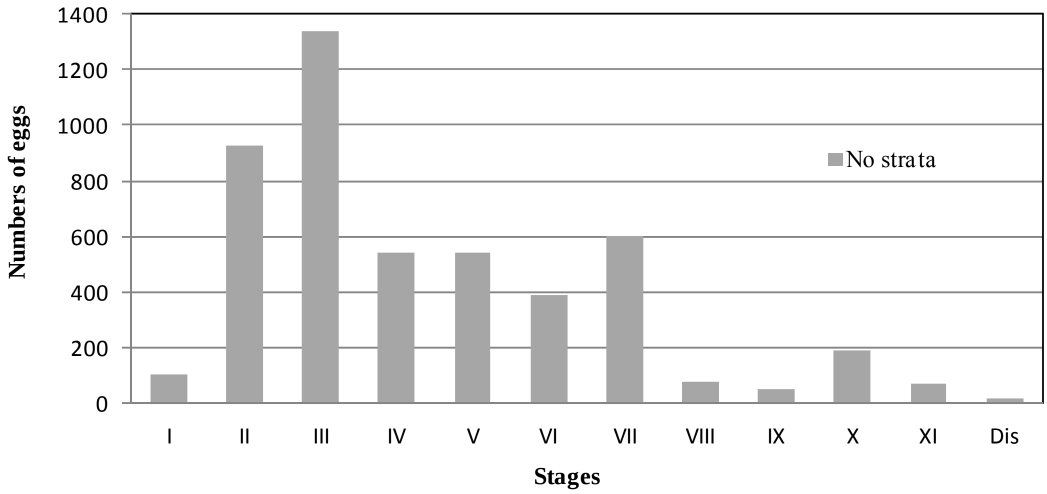


Figure 4. Eggs per stage of development from CalVET surveying (total eggs) by Stratum above (South, West and North) and no strata (below).

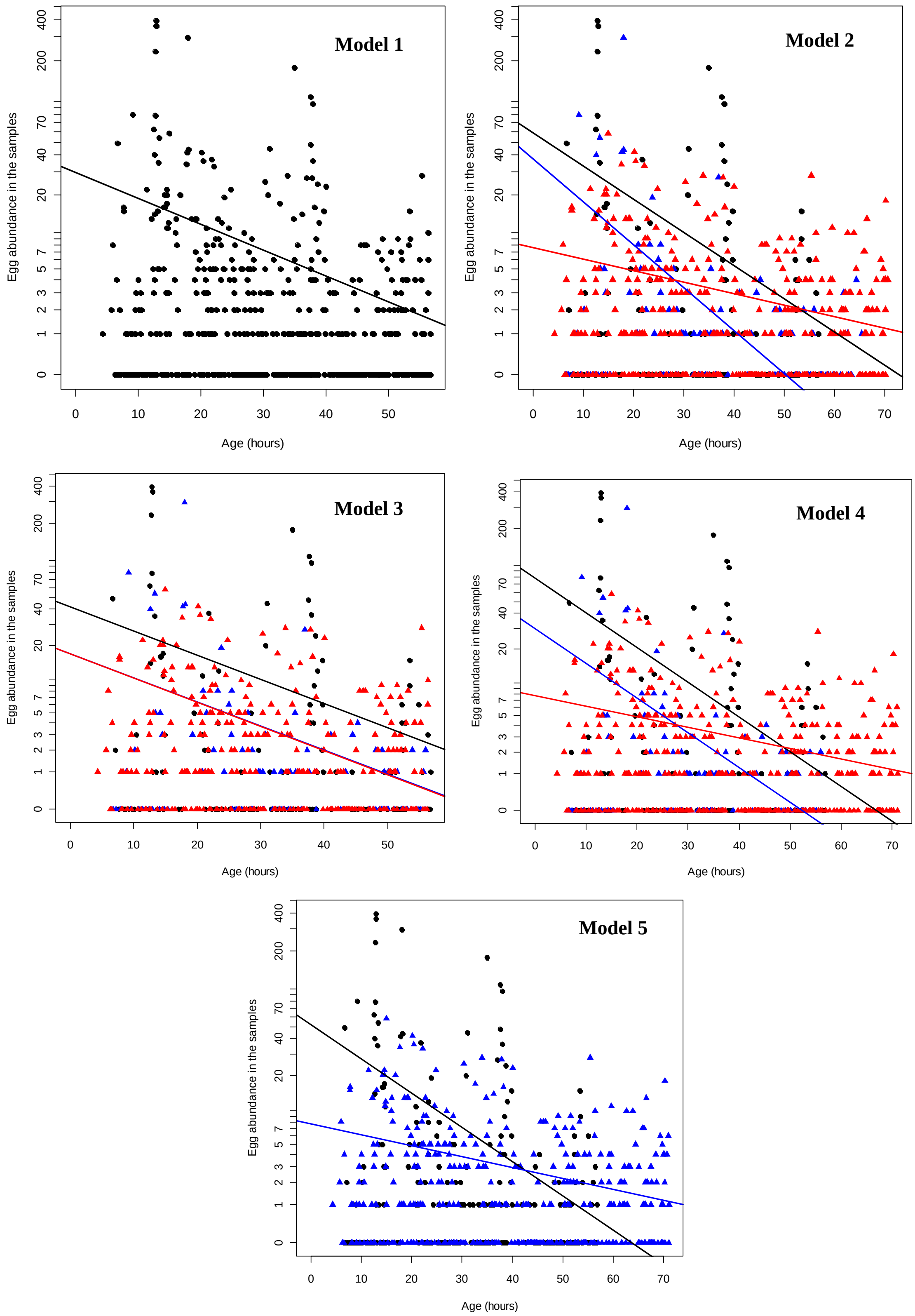


Figure 5. Abundance by age of eggs in the different spatial strata (black = south, blue = west, red = north) and its corresponding fitted mortality curve. Note that southern, western and northern mortality curves were forced to have a common slope (mortality) in Model 3. In Model 4, southern and western mortality curves were forced to have a common slope and that duration of the egg phase is larger in the northern stratum, due to lower temperatures. Below abundance by age of eggs in model 5 (black= south and west, blue= north) and the resultant fitted mortality curve.

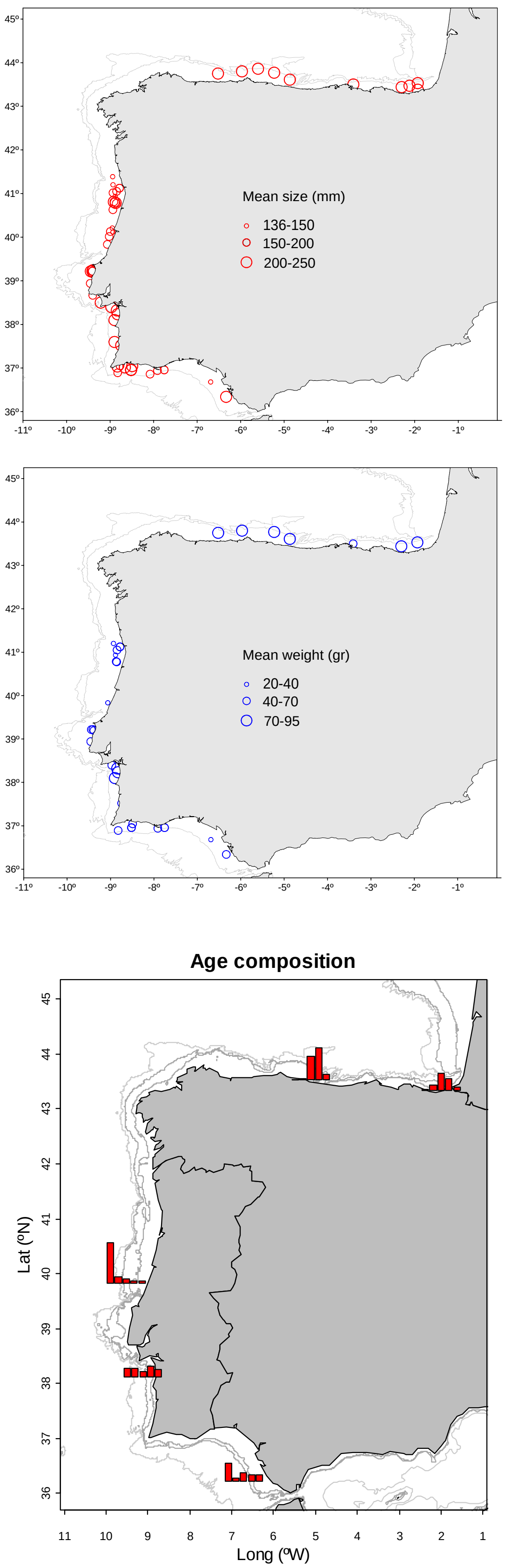


Figure 6. Spatial distribution of the mean size (above), mean weight for mature females, and age composition for the South, West and North areas.

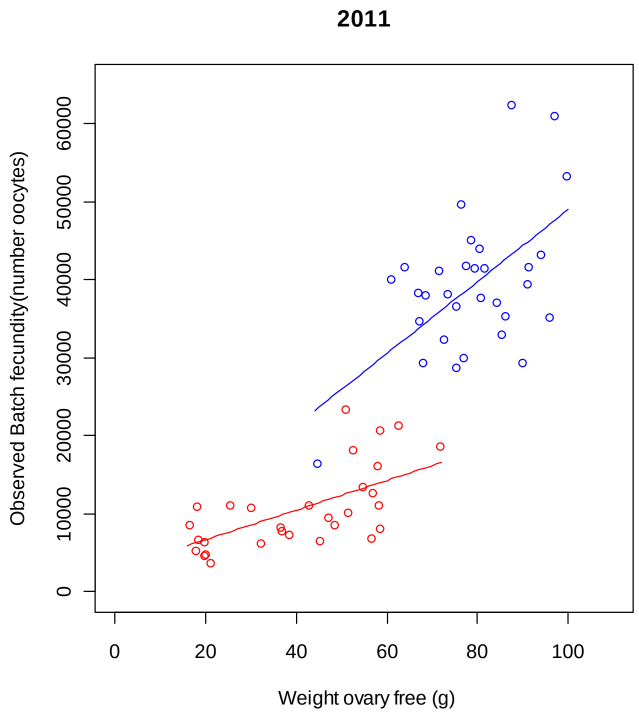


Figure 7. Observed batch fecundity vs. gonad free weight of the hydrated females and regression line of the corresponding model for two geographical areas (red: South and West strata, blue: North stratum).

Annex 1. The summaries of the five different models used to estimate egg production.

Model 1

```
Call:
glm.nb(formula = cohort ~ offset(log(Efarea)) + age, data = aged.data,
    weights = Rel.area, init.theta = 0.236726029864412, link = log)

Deviance Residuals:
     Min        1Q    Median       3Q       Max
-1.91987  -1.06620  -0.63781  -0.08508   2.93385

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  5.68829    0.23113  24.610  < 2e-16 ***
age         -0.04376    0.00669  -6.542 6.08e-11 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(0.2367) family taken to be 1)

    Null deviance: 496.29  on 553  degrees of freedom
Residual deviance: 455.47  on 552  degrees of freedom
AIC: 2251.3
Number of Fisher Scoring iterations: 1
              Theta:  0.2367
          Std. Err.:  0.0183
 2 x log-likelihood:  -2245.3110
```

Model 2

```
Call:
glm.nb(formula = cohort ~ offset(log(Efarea)) - 1 + Stratum +
    Stratum:age, data = aged.data, weights = Rel.area, init.theta = 0.264980200751917,
    link = log)

Deviance Residuals:
      Min         1Q     Median         3Q        Max
-2.066790  -1.056559  -0.606537  -0.003712   3.205770

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
Stratum1      6.375637   0.407038  15.663  < 2e-16 ***
Stratum2      5.928648   0.443085  13.380  < 2e-16 ***
Stratum3      4.914646   0.262852  18.697  < 2e-16 ***
Stratum1:age -0.056178   0.011968  -4.694 2.68e-06 ***
Stratum2:age -0.072667   0.011819  -6.148 7.83e-10 ***
Stratum3:age -0.019603   0.006165  -3.180  0.00147 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(0.265) family taken to be 1)
```

```
    Null deviance: 9811.67  on 654  degrees of freedom
Residual deviance:  531.18  on 648  degrees of freedom
AIC: 2545.5

Number of Fisher Scoring iterations: 1
              Theta:  0.2650
          Std. Err.:  0.0195
 2 x log-likelihood:  -2531.4690
```

Model 3

```
Call:
glm.nb(formula = cohort ~ offset(log(Efarea)) - 1 + Stratum +
    age, data = aged.data, weights = Rel.area, init.theta = 0.243416318891423,
    link = log)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-1.95719  -1.07565  -0.65446  -0.04393   3.61421

Coefficients:
          Estimate Std. Error z value Pr(>|z|)
Stratum1  6.036392   0.273268  22.090  < 2e-16 ***
Stratum2  5.134344   0.296519  17.315  < 2e-16 ***
Stratum3  5.706742   0.247276  23.078  < 2e-16 ***
age      -0.044800   0.006624  -6.763 1.35e-11 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(0.2434) family taken to be 1)

    Null deviance: 8905.45  on 554  degrees of freedom
Residual deviance:  455.44  on 550  degrees of freedom
AIC: 2245.4

Number of Fisher Scoring iterations: 1
              Theta:  0.2434
          Std. Err.:  0.0189
 2 x log-likelihood:  -2235.4400
```

Model 4

```
Call:
glm.nb(formula = cohort ~ offset(log(Efarea)) - 1 + Stratum +
    StratumI:age, data = aged.data, weights = Rel.area, init.theta = 0.265492603659438,
    link = log)

Deviance Residuals:
      Min         1Q     Median         3Q        Max
-2.107061  -1.053380  -0.594004   0.003369   3.390103

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
```

```
Stratum1        6.670898   0.328053  20.335  < 2e-16 ***
Stratum2        5.701506   0.347264  16.418  < 2e-16 ***
Stratum3        4.921170   0.260476  18.893  < 2e-16 ***
StratumI1:age -0.065520   0.009065  -7.228 4.91e-13 ***
StratumI2:age -0.019843   0.006044  -3.283  0.00103 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(0.2655) family taken to be 1)

    Null deviance: 9823.25  on 640  degrees of freedom
Residual deviance:  525.44  on 635  degrees of freedom
AIC: 2530.7

Number of Fisher Scoring iterations: 1
              Theta:  0.2655
          Std. Err.:  0.0195
 2 x log-likelihood:  -2518.6510
```

Model 5

```
Call:
glm.nb(formula = cohort ~ offset(log(Efarea)) - 1 + StratumI +
    StratumI:age, data = aged.data, weights = Rel.area, init.theta = 0.257780921160634,
    link = log)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-2.08031  -1.05183  -0.61488  -0.03243   3.20327

Coefficients:
               Estimate Std. Error z value Pr(>|z|)
StratumI1      6.253312   0.311997  20.043  < 2e-16 ***
StratumI2      4.921065   0.264116  18.632  < 2e-16 ***
StratumI1:age -0.062306   0.009099  -6.848  7.5e-12 ***
StratumI2:age -0.019839   0.006126  -3.238  0.00120 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(0.2578) family taken to be 1)

    Null deviance: 9676.46  on 640  degrees of freedom
Residual deviance:  525.61  on 636  degrees of freedom
AIC: 2540.6

Number of Fisher Scoring iterations: 1
              Theta:  0.2578
          Std. Err.:  0.0188
 2 x log-likelihood:  -2530.6130
```